Tricomponent Fibrous Scaffolds with Dual Delivery of rhVEGF and rhBMP-2 for Bone Tissue Engineering

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Introduction: Electrospinning has been constantly used by many researchers for fabricating various tissue engineering scaffolds. Assisted by composite fiber electrospinning (Tong HW, Wang M, *J. Nanosci. Nanotechnol.*, 2007, 7:3834-3840) and emulsion electrospinning (Wang C, Wang M, *Adv. Mater. Res*, 2012, 410:98-101), bicomponent scaffolds comprising osteoconductive calcium phosphate (Ca-P)/PLGA nanocomposite fibers and osteoinductive recombinant human bone morphogenetic protein 2 (rhBMP-2)/PDLLA fibers were successfully made (Wang C, et al., *Proc. 9th WBC*, Chengdu, China, 2012, Paper #562), which showed much enhanced *in vitro* biological performance in recent studies. Vascularization is a critical issue in bone tissue engineering using electrospun scaffolds. Recombinant human vascular endothelial growth factor (rhVEGF), an angiogenic growth factor, can promote vascularization. In this investigation, angiogenic, osteoconductive and osteoinductive tricomponent scaffolds were produced via multi-source dual-power electrospinning (MSDP-ES) and were subsequently studied using various techniques.

Methods: Ca-P nanoparticles were made in-house. PLGA (LA:GA=50:50; M_w=120,000), PEG (M_w=6,000), rhBMP-2, rhVEGF and other chemicals were supplied by reputable manufacturers/suppliers. Using a MSDP-ES setup (Fig.1), tricomponent scaffolds consisting of angiogenic rhVEGF/(PLGA/PEG) fibers, osteoconductive Ca-P/PLGA fibers and osteoinductive rhBMP-2/PLGA fibers were constructed, with rhBMP-2/PLGA and rhVEGF/(PLGA/PEG) fibers being made via emulsion electrospinning. The ratio of three fibers in tricomponent scaffolds was controlled to be 1:1:1. Monocomponent scaffolds having only Ca-P/PLGA, rhBMP-2/PLGA, or rhVEGF/(PLGA/PEG) fibers were also produced. Using the PLGA/PEG polymer blend as fiber matrix for rhVEGF aimed to achieve sequential release of firstly rhVEGF and then rhBMP-2. The morphology and structure of fibers and mono- and tricomponent scaffolds were subsequently studied. The *in vitro* release behaviors of rhBMP-2, rhVEGF and Ca^{2+} ions from scaffolds were investigated using human BMP-2 ELISA kit assay, human VEGF kit assay and calcium bioassay kit.

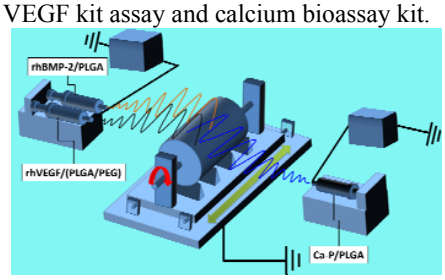

Fig.1. Multil-source dual-power electrospinning

Results: Fig.2 shows fibrous structures of mono- and tricomponent scaffolds and also the morphology of fibers in these scaffolds. Using the current MSDP-ES setup, rhVEGF/(PLGA/PEG), rhBMP-2/PLGA and Ca-P/PLGA fibers could be evenly distributed in tricomponent scaffolds. Under TEM examination, emulsion electrospun rhBMP-2/PLGA and rhVEGF/(PLGA/PEG) fibers in mono- and tricomponent scaffolds exhibited core-shell structures, with rhBMP-2 and rhVEGF molecules being contained (and hence protected) in the water phase core. Using our technology for composite fiber electrospinning, Ca-P particles were homogeneously distributed in fibers.

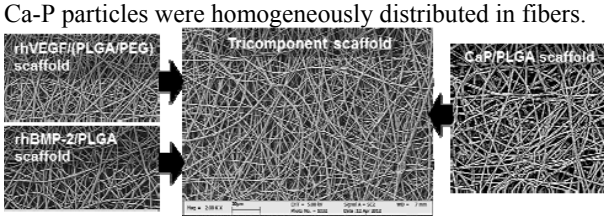

Fig.2. Mono- and tricomponent scaffolds

In vitro release behaviors of rhBMP-2 and rhVEGF for mono- and tricomponent scaffolds are shown in Fig.3. rhVEGF in (PLGA/PEG) fibers of mono- and tricomponent scaffolds exhibited similar release profiles, including a quick release in the initial 24 hr (up to the 30% and 25% level, respectively) and a sustained release afterwards. After 28 days, rhVEGF achieved the 70% and 82% release level, respectively. In comparison, rhBMP-2 in PLGA fibers of mono- and tricomponent scaffolds showed much reduced initial release (up to the 6% and 2% level, respectively). After 10 days, an accelerated release of rhBMP-2 was observed (Fig.3). Due to PLGA degradation in the test liquid (PBS with small amounts of additives), more pores were formed in fibers when immersion time increased. This had enhanced the diffusion of rhBMP-2 from fiber interior to the test liquid, promoting rhBMP-2 release. As PEG is a water soluble polymer, PLGA/PEG polymer blend degraded faster than pure PLGA, thus starting significant rhVEGF release earlier than rhBMP-2 release for tricomponent scaffolds. Ca^{2+} ions also exhibited the sustained release behavior within the 28 day release test period.

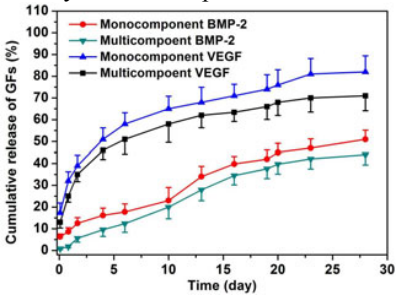

Fig.3 *In vitro* release profiles of rhBMP-2 and rhVEGF for mono- and tricomponent scaffolds

Conclusions: Tricomponent fibrous scaffolds for bone tissue engineering, with the fibers containing separately rhVEGF, rhBMP-2 and Ca-P nanoparticles, were successfully produced using MSDP-ES. Sequential delivery of two growth factors for different purposes could be achieved through the tricomponent scaffolds. The incorporation (and release) of rhVEGF should enhance vascularization of electrospun scaffolds.